

## **B-vitamin metabolism**

### ***Introduction***

B-vitamins - a group of water soluble vitamins including thiamine, riboflavin, niacin, B-6, B-12, folate, pantothenic acid and biotin - are present in a wide variety of foods. Daily intake of these vitamins is required for proper bodily function, and deficiency has been associated with a number of serious disorders/illnesses.<sup>1</sup>

Reports in the scientific literature have focused on the protective effects of B-vitamins, specifically B-6, B-12, and folate, against certain disorders and illnesses. Even modest deficiencies have been associated with diseases such as neural tube defects, cardiovascular disease and cancer.<sup>2</sup> These vitamins are critical for cellular function, playing a crucial role in DNA repair, detoxification of reactive biochemistry by the liver and growth of new cells.

A vital function of vitamins B-6, B-12 and folate is the key role they play in one-carbon metabolism (Figure 1), required for the de novo synthesis of purines (the building blocks of RNA and DNA) and the methylation of homocysteine to form methionine. Methionine (an essential amino acid), which is converted to S-adenosylmethionine (SAM), acts as a universal donor of methyl groups and is required for synthesis of DNA, RNA, hormones, neurotransmitters, membrane lipids, proteins and others.<sup>3</sup>

In relation to one-carbon metabolism, folate is a precursor of tetrahydrofolate (THF); transcobalamin 2 (TCN2) is a supplier of B-12, which is a cofactor for the enzyme methionine synthase (MS), that transfers a methyl group (from 5-methyltetrahydrofolate ((5-TMHF)) to homocysteine (Hcy) forming methionine; B-6 is a co-factor for cystathionine  $\beta$ -synthase (CBS) and is critical for degrading Hcy. Thus, B-6, B-12 and folate are all involved in regulating Hcy levels. Proper functioning of this pathway prevents the buildup of Hcy, which has been linked to cellular dysfunction and disease.

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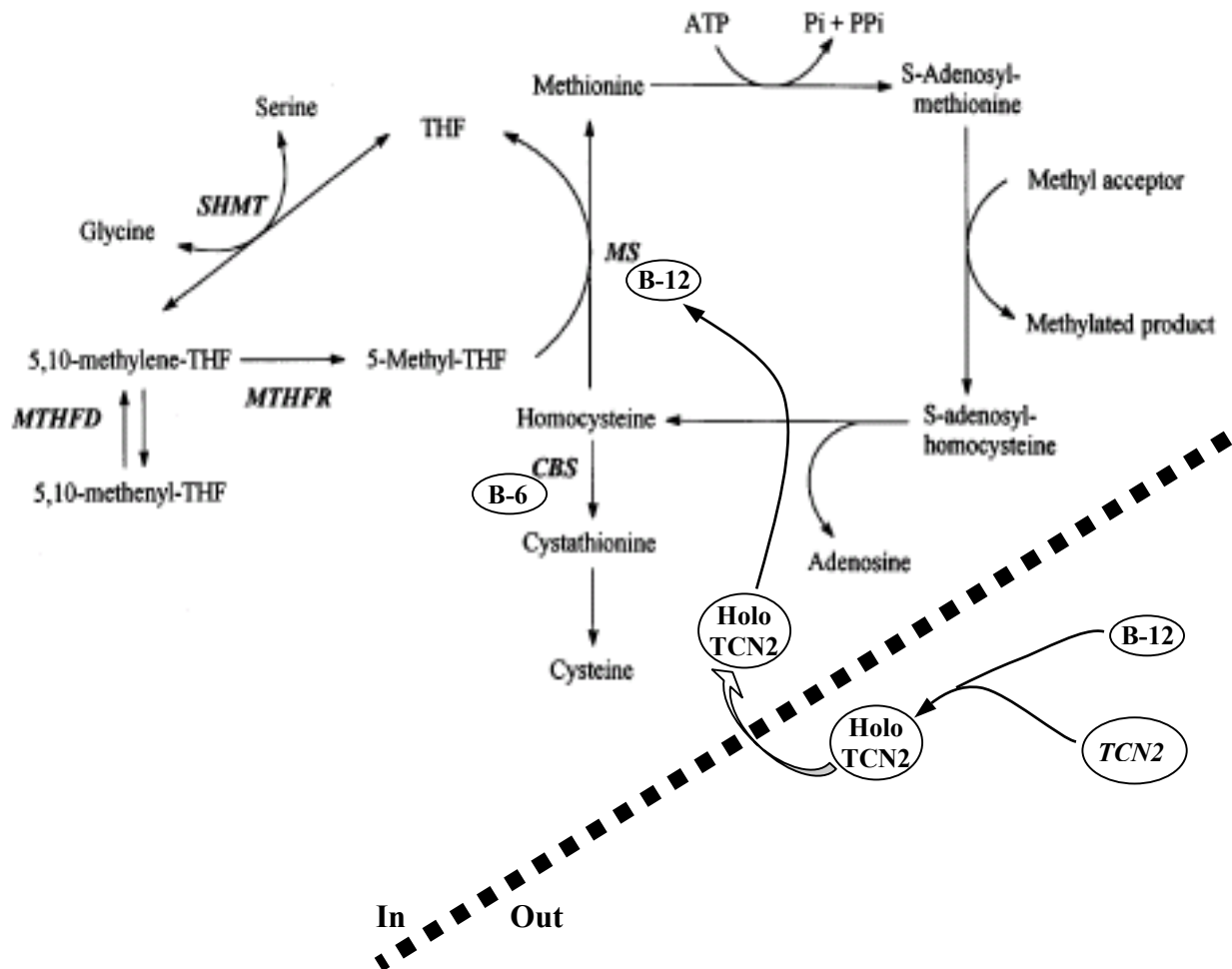


Figure 1: A simplified scheme of one-carbon metabolism. Folate is a precursor of tetrahydrofolate (THF), B-12 is a cofactor for the enzyme methionine synthase (MS), and B-6 is a co-factor for cystathionine  $\beta$ -synthase (CBS). Transcobalamin 2 (TCN2) is a transport protein, binding to B-12 in the plasma to form holo-TCN2. Holo-TCN2 is recognized and taken up by specific receptors on cells. MTHFR, 5,10-methylenetetrahydrofolate reductase; MTHFD, 5,10-methylenetetrahydrofolate dehydrogenase; SHMT, serine hydroxymethyl transferase; <sup>3-6</sup>

Genetic variations in 5,10-methylenetetrahydrofolate reductase (MTHFR) or TCN2 may prevent appropriate utilization of vitamins B-6, B-12 and folate in one-carbon metabolism. Evidence suggests that the presence of *TCN2* 776C>G or *MTHFR* 677C>T variant genotypes disrupts Hcy metabolism as well as the methylation of proteins, DNA and phospholipids in cells and is associated with certain disease states. Studies have explored the possibility that increasing plasma levels of vitamins B-6, B-12 and folate will benefit persons carrying these genetic variations.

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**Summary of genetic variations**

	<b>Polymorphism</b>	<b>Biochemical impact of variant</b>	<b>Variant affects disease risk</b>	<b>Nutritional modification</b>
MTHFR	<i>MTHFR</i> Ala 222 Val (677C>T)	Dysfunction leads to impaired folate metabolism, required in conjunction with co-factor vitamin B-12 for one-carbon metabolism	Disrupts Hcy metabolism, methylation of proteins, DNA and phospholipids May increase risk of disorders associated with elevations in Hcy levels, cardiovascular disease, neural tube defects	<p>Implicated in coronary heart disease, colon cancer and NTD in the setting of B-vitamin deficiency. Sources of B vitamins in the diet include fortified cereals, flours, pastas, rice and other grains, leafy green vegetables, dried beans, peas, fruits (eg, orange juice), and animal foods including fish, meat, poultry, eggs, milk and milk products.</p> <p>Folate supplementation in mothers reduced risk for high level neural tube defects in offspring with TT variant</p> <p>Insufficient folate intake periconceptionally in mothers carrying TT genotype increased occurrence of cleft lip (with or without cleft palate) in offspring</p>
TCN2	<i>TCN2</i> Pro 259 Arg (776C>G)	Impaired binding and transport of vitamin B-12 into cells Deficient B-12 levels for one-carbon metabolism	Disrupts Hcy metabolism, methylation of proteins, DNA and phospholipids May increase risk of disorders associated with elevations in Hcy levels, such as cardiovascular disease, Alzheimer's disease, neural tube defects	<p>Implicated in heart disease, Alzheimer's disease and neural tube defects depending on vitamin B-12 and Hcy status. Sources of vitamin B-12 in the diet include fortified cereals and animal foods including fish, meat, poultry, eggs, milk and milk products.</p>

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### ***MTHFR genetic variation***

#### **Individuals with genetic variation in MTHFR demonstrate differences in metabolic responses to vitamins and other nutrients**

The variant of the gene encoding MTHFR – *MTHFR* 677C>T – causes an alanine-to-valine amino acid substitution in the folate binding site. This results in a lowered enzymatic activity: mean MTHFR activity in individuals with the TT genotype has been reported as approximately 30% of that measured in CC individuals ( $P < 0.01$ ).<sup>7</sup> Mean MTHFR activity in heterozygotes was 65% compared with CC individuals.<sup>7</sup>

Knock-out studies of *MTHFR* in mice indicate that functional MTHFR levels are a determinant of Hcy concentrations, with a 10-fold increase in Hcy concentration detected in homozygotes (-/-; both chromosomes lack *MTHFR*), and a 1.6-fold increase in heterozygotes (+/-; one chromosome lacks *MTHFR*) over wild-type littermates (+/+) ( $P < 0.05$ ).<sup>8</sup> Clinically, the association between Hcy levels and MTHFR genotype is more complex and is influenced by a number of subject-specific variables including B-12 and folate status, gender and age.<sup>9</sup> While a significant increase in Hcy concentration under low plasma folate conditions was detected in TT individuals compared with CT and CC genotypes ( $< 12.5$  nmol/L) ( $P = 0.001$ ), this relationship was not apparent among those with higher folate status.<sup>9</sup> Further stratification revealed an age and gender interaction ( $P = 0.002$ ), with a significant association between genotype and Hcy concentration in males  $< 55$  years old ( $P < 0.001$ ).<sup>9</sup> An interaction between B-12 status and genotype has also been reported, with low plasma B-12 concentrations ( $< 148$  pmol/mg) in individuals with the TT variant leading to significantly higher Hcy concentrations, compared to individuals with vitamin B-12 concentrations within the normal range ( $\geq 148$  pmol/mg).<sup>10</sup>

#### **Studies indicate that individuals with variation in MTHFR genotype may be at increased risk for certain disorders or illnesses, particularly in the setting of B-vitamin deficiency**

**Cardiovascular disease:** Three separate meta-analyses performed on data from clinical studies have detected associations between variant genotypes of MTHFR and cardiovascular disease.<sup>11-13</sup> One such meta-analysis (Klerk et al., 2002) of 40 observational studies (11,162 cases and 12,758 controls) determined a 16% elevated risk of coronary heart disease (CHD) in individuals with the *MTHFR* 677 TT genotype compared with the CC genotype (Odds Ratio (OR) = 1.16, 95% confidence interval (CI): 1.05-1.28).<sup>11</sup> A meta-analysis (Kluijtmans et al., 2001) of ten studies (1,857 cases and 2,842 controls) reported a significantly increased atherothrombotic risk in TT genotype patients, and an increased risk for atherothrombotic disease was also observed in heterozygotes (OR = 1.27, 95% CI: 1.11-1.44).<sup>12</sup> However, a third meta-analysis (Brattstrom et al., 1998) was unable to identify an increased risk for cardiovascular disease associated with the TT genotype following the analysis of 23 case-control studies comprising 5,869 patients and 6,644 controls.<sup>13</sup>

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Those bearing the TT genotype did, however, have plasma Hcy concentrations 2.6µmol/L (25%) higher than those with the CC genotype.<sup>13</sup> Based on findings of these meta-analyses, individuals with the TT genotype have elevated risk of hyper-homocysteinemia and cardiovascular disease.

**Colorectal cancer (CRC):** There are conflicting findings regarding the association between MTHFR genotype and risk of CRC.<sup>14</sup> While some reports have determined that CRC risk is decreased in carriers of the *MTHFR* 677 T gene,<sup>3, 15-17</sup> others have indicated no significant association or possibly even an increased risk.<sup>18, 19</sup> Although there have been reports of interactions between CRC risk genotypes and B6, B12 status<sup>1, 41, 43</sup>, the preponderance of results associating MTHFR genotype with CRC risk have not been consistent enough to draw definitive conclusions concerning interpretation of this test with respect to CRC risk.

**Neural Tube defects:** There is considerable evidence suggesting a significant association between *MTHFR* polymorphisms and NTD. In a Dutch meta-analysis of 1,273 individuals, the TT genotype was present in 8.4% of the study population.<sup>20</sup> Presence of the TT genotype was associated with an increased risk for NTD in mothers (OR = 1.9, 95% CI: 1.1-3.1), and patients (OR = 1.5, 95% CI: 0.74-3.1). A study of 175 American-Caucasians with NTD and their families, and 195 unrelated controls, reported an increased risk for NTD in TT patients (OR = 2.13, 95% CI: 1.11-4.09) but not in mothers or fathers.<sup>21</sup> Similar results have been reported in other studies.<sup>22, 23</sup> Although a small French study of 43 cases of myelomeningocele spina bifida or anencephaly failed to find a significant correlation, the study was investigating severe cases of NTD that were prenatally diagnosed rather than in persons born with the defect.<sup>24</sup> A significant interaction between genotype and folate status has also been reported relative to NTD:<sup>23</sup> under conditions of low folate levels, persons homozygous for the *MTHFR* TT genotype were at greater risk for NTD, with an OR of 13.43 (95% CI: 2.49-72.33) for patients, and 3.28 (95% CI: 0.84-12.85) for mothers.<sup>23</sup>

### **People with variation in MTHFR genotype at risk of associated disorders/illnesses may benefit from nutritional modification to increase intake of folate and/or vitamin B-12.**

Several studies have investigated the association between plasma levels of folate and/or B-12 levels and health risks in individuals with MTHFR variant genotypes.<sup>9, 11, 13, 23</sup>

Generally, evidence suggests that homozygosity for *MTHFR* 677C>T variants is associated with increased Hcy concentrations under conditions of low B-12 and/or folate status (see Table below). This interaction is not present at higher levels of these vitamins, which may confer protection against Hcy accumulation and certain diseases.<sup>25, 26</sup> Based on the studies shown in the table below, diets enriched in folate and B-12 may benefit individuals with *MTHFR* TT homozygous genotypes.

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<b>Gene SNP</b>	<b>Genotype</b>	<b>Vitamin/nutrient concentration</b>	<b>Effect of vitamin/nutrient concentration on Hcy and/or risk of disease/disorder</b>	<b>Publication</b>
<i>MTHFR</i> 677C>T	TT	Plasma folate levels < 12.5 nmol/L	Significantly increased Hcy concentration compared with CT and CC genotypes ( $P = 0.001$ ). Difference not present at higher folate levels.	9
	TT	Plasma folate levels below the median or in the lowest quartile	Significantly increased Hcy concentration compared with CT and CC genotypes. Difference not present at higher folate levels.	13
	TT	Plasma B-12 levels < 148 pmol/mg	Significantly increased Hcy concentration compared with normal levels ( $\geq 148$ pmol/mg) of vitamin B-12.	10
	TT	Low folate status	Significantly increased CHD risk compared with CC genotype only present when folate status is low.	11
	TT	RBC folate levels in the lowest quartile	Significantly increased risk of NTD (case or case mother) compared with risk associated with TT genotype or folate level alone.	23

Interaction between vitamin levels and genotype on Hcy concentration and/or disease risk

Maintaining adequate levels of B vitamins is possible through a well-balanced diet (see Table below), although the majority of people fail to optimize vitamin consumption through diet alone.<sup>27</sup> Enrichment of foods has helped in this goal, for example the mandatory introduction in 1998 of grain products fortified with folate in the US.<sup>28</sup> Vitamin supplementation has also been shown to effectively decrease Hcy concentrations,<sup>29-31</sup> as has consumption of fortified breakfast cereals.<sup>32</sup>

<b>B-vitamin</b>	<b>Dietary sources</b>
Folate	Enriched breads, cereals, flours, corn meals, pastas, rice and other grains. Leafy green vegetables, dried beans and peas, fruits (eg, orange juice).
Vitamin B-6	Fortified cereals, beans, meat, poultry, fish and some fruits and vegetables
Vitamin B-12	Fortified cereals and animal foods including fish, meat, poultry, eggs, milk and milk products.

Dietary sources of folate and vitamins B-6 and B-12

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Supplementation with folic acid has proven effective for preventing first occurrences of neural tube defects (NTDs) as well as recurrences in individuals with a history of NTD births.<sup>33,34</sup> In a study of 145 infants with spina bifida (SB) and 260 non-malformed control infants, infants with the *MTHFR* 677TT genotype were at greater risk of having high level defects (OR = 5.5, 95% CI: 0.8-28.1) than low level defects (OR = 1.8, 95% CI: 0.8-3.9) when their mothers did not use vitamin supplements containing folic acid.<sup>26</sup> A case-control study in the Netherlands, involving 179 cases of cleft lip with or without cleft palate (CLP) and 204 controls, revealed an increased prevalence of CLP in children when mothers homozygous for the *MTHFR* 677TT genotype failed to take folic acid supplements periconceptionally (OR = 5.9, 95% CI: 1.1-30.9), had a low dietary folate intake (OR = 2.8, 95% CI: 0.7-2.5), or both (OR = 10, 95% CI: 1.3-79.1).<sup>25</sup>

### The GNP provides a risk assessment tool that may be used to guide some nutritional and lifestyle decisions intended to optimize wellness

The *MTHFR* 677T allele frequency differs between ethnic and racial groups:

Study population	<i>MTHFR</i> T allele frequency <sup>35</sup> (%)	% TT homozygotes (positive test result)
Britain	35.4	13.2
Ireland/N. Ireland	32.5	10.8
France	36.1	9.8
Germany	24.5	7.8
Italy	43.8	18.0
The Netherlands	32.2	8.9
Norway	28.0	9.5
Sweden	30.2	10.3
Sub-Saharan Africa	6.3	0.0
Yemen	17.4	2.2
Turkey	28.5	7.5
Asia	17.6	3.5
Japan	35.2	11.5
Australia	35.8	10.7
Brazil, Whites	37.4	10.3
Brazil, Blacks	17.9	1.6
Brazil, Amerindians	11.4-44.9	1.2-20.5
Colombia	48.7	25.3
Canada (Quebec)	36.3	14.3
US, Whites	34.2	11.9
US, Blacks	14.0	1.2
US, Hispanics (CA)	41.7	20.7

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### ***TCN2 genetic variation***

#### **Individuals with genetic variation in TCN2 demonstrate differences in metabolic responses to vitamins and other nutrients**

Transcobalamin 2 is a transport protein that specifically binds vitamin B-12 to form holo-TCN2 making it available for cellular uptake.<sup>10</sup> The gene coding for TCN2 (*TCN2*) is subject to a number of genetic variations, of which the common polymorphism *TCN2* 776C>G results in a proline-to-arginine amino acid substitution at codon 259 (P259R) of the TCN2 protein.

Studies suggest that the arginine form of the protein is less effective at binding B-12 than the proline form, potentially leading to increased Hcy. Individuals with the GG genotype tend to have lower holo-TCN2 concentrations compared with CC individuals, despite similar total serum B-12 concentrations ( $P < 0.05$ ).<sup>10, 36</sup> The GG genotype is also associated with lower TCN2 saturation (holo-TCN2/total TCN2) compared with GC and CC genotypes in individuals with similar total plasma vitamin B-12 concentrations.<sup>10</sup> A decrease in holo-TCN2 saturation may potentially result in a decreased cellular B-12 concentration and consequently an accumulation of Hcy.<sup>6, 37</sup> Low holo-TCN2 concentrations have been associated with significantly higher mean plasma Hcy concentrations in individuals with the GG genotype,<sup>10</sup> as have low TCN2 saturation ratios in individuals with low nutritional vitamin B-12 status.<sup>6</sup> In a study of dementia patients and healthy elderly volunteers, median serum Hcy concentration was higher in GG than in CC individuals, with Hcy concentration in heterozygotes intermediate, although this trend failed to achieve statistical significance ( $P = 0.09$ ).<sup>38</sup>

#### **Studies indicate that individuals with variation in TCN2 genotype may be at increased risk for certain disorders or illnesses, particularly in the setting of B-vitamin deficiency**

**Cardiovascular disease:** Meta-analyses have identified raised Hcy concentrations as an independent risk factor for CVD.<sup>39-41</sup> One study of 190 vascular disease patients and 601 healthy controls, found no association between *TCN2* polymorphisms and cardiovascular disease. However, when the results were stratified according to B-12 concentration, an association emerged between genotype and plasma Hcy concentration.<sup>42</sup> Hcy concentrations in individuals in the highest quartile for B-12 concentration ( $> 299\text{pmol/L}$ ) were lower in CC than in GG individuals or heterozygotes ( $P$  by ANOVA  $< 0.01$ ).<sup>42</sup> Further research is warranted to determine if a relationship exists between *TCN2* genotype and cardiovascular risk.

**Alzheimer disease (AD):** A prospective study of 1,092 subjects (667 women and 425 men; mean age 76 years) revealed a strong association between Hcy and risk for AD. Disease risk was nearly doubled in individuals with mild-to-moderately elevated plasma Hcy concentrations ( $> 14\mu\text{mol/L}$ , relative risk (RR) = 1.9, 95% CI: 1.2-3.0).<sup>43</sup> Another study of 180 AD cases and 181 controls reported similar results: patients with AD had higher Hcy concentrations than the control group ( $P = 0.0231$ ) and those with Hcy concentrations in the upper tertile were significantly more likely to develop AD (OR = 2.8, 95% CI: 1.54-5.22,  $P = 0.0008$ ).<sup>44</sup> *TCN2*

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genotype was not directly associated with AD risk.<sup>44</sup> However another case-controlled study reported significantly more GG individuals appeared to develop AD at any age compared with CC individuals ( $P = 0.008$ ).<sup>45</sup> Further studies may be required to reconcile these differences.

**Neural Tube defects:** A case-control study of 103 mothers of children with spinabifida and 100 adult controls reported a significantly higher prevalence of the *TCN2* 776 GG genotype in mothers of children with neural tube defects (NTDs) (OR = 2.22, 95% CI: 1.1-4.5,  $P = 0.022$ ).<sup>37</sup> A smaller study (46 mothers of children with NTD and 73 female controls) failed to demonstrate an association between genotype and risk for NTD, but did report that case mothers were significantly more likely to have holo-TCN2 levels (TCN2 bound to vitamin B-12) and saturation ratios (holo-TCN2/total TCN2) within the lowest, rather than highest quartile ( $P = 0.04$  and  $P = 0.03$  for holo-TCN2 levels and saturation ratios respectively).<sup>6</sup> These authors suggest that B-12 supplementation, in addition to folate supplementation, may be beneficial in prevention of NTDs.

### People with variation in *TCN2* genotype at risk of associated disorders/illnesses may benefit from nutritional modification increasing intake of vitamin B-12.

Reports suggest that, regardless of *TCN2* genotype, increased vitamin B-12 status is associated with decreased Hcy levels.<sup>6, 10</sup> However, this may be particularly important for individuals with variation in *TCN2* (776C>G), which results in less efficient binding to B-12 for transport into cells,<sup>10, 36</sup> and may lead to elevations in Hcy levels.<sup>6, 37</sup> In one such study, significantly raised Hcy concentrations were detected in carriers of the variant *TCN2* G allele compared with the CC genotype, despite vitamin B-12 levels falling within the highest quartile (> 299 pmol/L) ( $P < 0.01$ ).<sup>42</sup> Consumption of foods enriched in vitamin B-12 may be particularly important for those individuals with genetic variations of *TCN2* whose ability to bind, and transport vitamin B-12 into cells, is impaired. Dietary sources of vitamin B-12 include fortified cereals and animal foods including fish, meat, poultry, eggs, milk and milk products.<sup>6, 10</sup>

### The GNP provides a risk assessment tool that may be used to guide some nutritional and lifestyle decisions intended to optimize wellness

The *TCN2* (776C>G) variant genotype is common in many ethnic and racial groups:

Study population	G allele frequency (%)	% GG homozygotes (positive test result)
Canada, Caucasians (Ontario) <sup>46</sup>	44	20.0
Sicily <sup>47</sup>	33.8	11.4
US, Columbia <sup>36</sup>	45	20.3
US, Black <sup>46</sup>	36	10.0
Sweden <sup>48</sup>	37	13.7
UK <sup>48</sup>	38	14.4
Germany <sup>49</sup>	41.2	17.0
Canada, Asians (Ontario) <sup>46</sup>	56	28.0

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### **Conclusion**

Vitamins B-6, B-12 and folate are co-factors essential for one-carbon metabolism and critical for regulating Hcy levels in cells. Variations in genotypes for *TCN2* and *MTHFR* compromise the appropriate utilization of these vitamins within the one-carbon metabolic pathway. This leads to accumulation of homocysteine in cells, and impaired methylation of cellular constituents.

Genetic variation in *TCN2* (776C>G) has been implicated in heart disease, Alzheimer's disease and neural tube defects depending on vitamin B-12 and Hcy status. Variation in *MTHFR* (677C>T) has been implicated in coronary heart disease, colon cancer and NTD in the setting of B-vitamin deficiency. *TCN2* and *MTHFR* variations are common in various regions around the world, and among persons of diverse ethnic and racial backgrounds.

Nutritional modification may help compensate for decreased utilization of vitamin B-6, B-12 and folic acid in individuals with *TCN2/MTHFR* variant genotypes. Increasing B-12/folate status may help reduce Hcy levels as well as disease risk. One of several examples is in the case of NTD, where a significant interaction between genotype and folate status was reported. Determining an individual's vitamin requirements based on genotype, and maintaining optimal levels of these vitamins may improve outcome in individuals carrying these variants. Use of nutritional supplements, along with ingestion of well-balanced meals that include fortified foods, are ways of optimizing vitamin intake based on genotypic requirements.

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